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Histochemistry with Labelled Antibody

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It has been known for some years that antibodies labelled with approximately two molecules of fluorescein per protein molecule can be used as specific fluorochromes for the localization of antigenic material in tissue cells.[1] After applying a labelled antibody solution to a tissue section for long enough to allow the labelled antibody to be bound by the antigen present, then washing off the excess, the fluorescent protein deposit can be visualized under the fluorescence microscope.

Recent modifications in this method by means of layers have allowed the demonstration of antibody in the cells of immune animals. When sections of lymphoid tissue from such animals are exposed to a dilute solution of the specific antigen, antigen is bound by the antibody in the tissue cells. If excess antigen now be washed away, the deposited antigen can then be detected by specific labelled antiserum, and hence the antibody present in the tissue cells visualized. Another use of layers has also been developed. When unlabelled specific antiserum is placed over a tissue section containing antigen, the globulin molecules are deposited from the serum over the sites of antigen localization. After excess unbound serum has been washed away, these globulin deposits can be detected by means of labelled specific anti-globulin serum.[2] Slides illustrating the application of these methods to the detection of polysaccharide and protein antigens injected into animals, antibody in the cells of immune rabbits, and viruses in infected cells, both in susceptible animals and in tissue cultures were shown.

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Mixed Infections with Influenza and Mumps Viruses in the Chick Embryo

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The use of fluorescent antibody in the localization of influenza and mumps viruses following intra-amniotic inoculation into the developing chick embryo[1, 2] has shown that the multiplication of these agents is restricted to the cells lining the amniotic cavity and the epidermal and pharyngeal epithelium. However, while the infection with the influenza virus is characterized by a diffuse type of staining which is first detectable in the nuclei and later in the cytoplasm of the cells, the growth of mumps virus takes place in discrete, multiple foci primarily within the cytoplasm of the cells. To determine whether this difference in localization would permit the viruses to occupy the same cells simultaneously, as has been suggested by the work of Ginsberg, Goebel and Horsfall,[3, 4] these experiments were extended to a study of mixed infections.

The viruses used consisted of the Enders' strains of influenza and mumps viruses passaged serially in eggs. Groups of seven to eight day old embryos were injected into the amniotic sac simultaneously or at intervals with varying amounts of both viruses while the controls received similar injections with either one or the other virus at the appropriate time interval. The eggs were incubated from one to three days and the mortality recorded. Frozen sections were made from the tissues of a representative number of survivors and pairs of consecutive sections were stained with either one or the other homologous labelled antiserum and in some instances with both antisera in succession. The stained preparations were examined under the fluorescence microscope and when necessary photographed.

The results obtained showed that when the viruses were injected simultaneously and the amount of PR8 virus used was large, most of the embryos either died or showed the presence of only the PR8 virus on the third day of incubation irrespective of the size of the mumps inoculum. If, however, the amount of PR8 virus used was very small as compared to mumps virus or if the embryos were examined on the first day of incubation, the amniotic membranes showed varying degrees of involvement with both viruses. In many of the areas, moreover, the viruses appeared to occupy the same cells.

When the mixed infections were produced at intervals, and the influenza virus was given within the first 24 hours after the injection of mumps virus, the results were essentially similar to those of the simultaneous infections. As the interval between the inoculation of the two agents was prolonged to one and two days, however, the infection with mumps virus assumed the ascendancy in direct relation to the size of the inoculum. The staining seen under these conditions showed the presence of varying numbers of cells which appeared to be infected with both viruses, particularly in those areas in which the infection with mumps virus was at an early stage. Finally, by spacing the two injections three days apart or longer, the multiplication of mumps virus became sufficiently advanced, with even the smallest inocula used, to exclude most or all of the PR8 virus injected at this time.

It is, therefore, apparent that the outcome of mixed infections with the viruses of influenza and mumps is governed by the factors of the amounts of each virus used, the interval between injections and the relative rates of growth of the two agents.

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Studies on Primary Atypical Pneumonia

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Some uncertainty has surrounded the identity of the etiological agent of Primary Atypical Pneumonia (PAP), although experimental transmission of the disease to human volunteers[1] suggested that the causative agent is probably a filtrable virus. Current methods for the diagnosis of PAP depend on the clinical picture of the illness, x-ray findings of the lungs and the development of cold agglutinins or streptococcus MG agglutinins in the sera of patients convalescent from the illness.

In 1944, an agent which was transmissible to cotton rats, hamsters, and chick embryos was isolated from PAP patients by Eaton, Meiklejohn and van Herrick.[2] The agent, designated as the Mac strain of PAP virus, has since been propagated in chick embryos for more than 60 serial passages. Suspensions of lung, trachea, and amniotic membranes from infected embryos induce pneumonia in cotton rats by intranasal instillation.

In the present studies of Primary Atypical Pneumonia, the indirect fluorescent staining method as described by Dr. Coons was used. Convalescent sera from human cases of PAP and anti-human globulin fluorescent rabbit sera were employed to detect the localization of viral antigen in infected chick embryo lungs. Furthermore, with known PAP infected chick embryo lung sections, the same procedure can be used to detect and titrate the antibody content of human sera for the serological diagnosis of PAP.

After inoculation of the Mac strain of PAP virus into the amniotic sac of chick embryos, the viral antigens were found to localize in the cytoplasm of bronchial epithelial cells. The infection was distributed primarily in the bronchi near the bifurcation of the trachea and in some of their larger branches. No viral antigens were demonstrated in the heart, the great vessels, or the amniotic membrane of the same infected embryos.

The age of the embryos was important for cultivation of the virus. When ten day old embryos were inoculated into the amniotic sac and incubated at 35°C for seven days, only 30-40 per cent showed viral growth in the lungs. But when 13 day old embryos were inoculated in the same manner and incubated for five days at 35°C, virtually 100 per cent of the inoculated eggs became infected.

Four new strains of PAP virus were isolated by inoculating sputa or lung suspension into the amniotic sac of 13 day old embryos. Three strains were isolated from the sputa of patients during the acute stage of the illness. The fourth strain was isolated from the lung of a patient who died in 1943. This lung was frozen at –70°C since autopsy, and kindly made available to us by Dr. Maxwell Finland. The evidence that they were probably agents from PAP is: 1) These agents react with human convalescent sera but not with acute phase sera from PAP. 2) The growth and localization of the virus in chick embryos are similar to the Mac strain of PAP virus. 3) The agents produce pneumonia in cotton rats when inoculated intranasally, and this pneumonia can be neutralized by human convalescent serum. 4) Antigenically they all cross-react including the Mac strain of virus.

In patients recovered from PAP infection, the antibody responsible for fluorescent staining appeared between the second and the third week of illness and all of them had antibody when the sera were collected three weeks after infection. The rise of antibody as measured by fluorescent staining and by cotton rat neutralization tests was parallel. Among seven patients so far studied, the fluorescent staining antibody has persisted in undiminished titers for three to ten months.

Agglutinins to human 0-type cells (cold

agglutinins) and to the non-hemolytic streptococcus MG frequently develop during the course of PAP infections.[3, 4] The cold agglutinin is unrelated to the PAP fluorescent staining antibody, because absorption of convalescent sera with human 0-type cells in the cold did not affect the fluorescent staining antibody titer. However, absorption of convalescent sera with streptococcus MG organisms did remove or diminish the fluorescent staining antibody titer. Yet rabbit serum with a high streptococcus MG agglutinin titer did not show fluorescent staining on infected chick embryo lung sections, nor did rabbits immunized with PAP virus develop MG agglutinins.

Of 44 PAP patients' sera collected during convalescence in the Boston area, about 70 per cent of them showed fluorescent staining antibody against PAP virus. Of 27 sera collected from characteristic cases of PAP from a private boys' school in New Hampshire, 100 per cent of them developed antibody against the virus. Most of these patients had cold agglutinins when tested. As to the specificity of the serological test for PAP, no cross reactions between influenza A and B infections, psittacosis, Q fever, or APC (Rowe, Huebner et al.) virus and the PAP virus have been encountered.

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The Effect of Colchicine on Antibody Production

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Recent findings have indicated that antibody is first detectable after antigenic stimulation in large undifferentiated cells lying in the medullary areas and around the follicles of the regional lymph nodes. These cells multiply and differentiate, meanwhile their antibody content increases, and by the end of the fourth or fifth day, their descendants form colonies of plasma cells in these regions. This description, which applies to the secondary antibody response, is quite different from the course of events following a first injection, for in this case very few antibody containing cells can be found.[1] Because of the evident dependence of antibody formation, particularly in the secondary response, on the multiplication of differentiating cells, it was thought that the manipulation of antibody response with so-called "mitotic poisons" would shed more light on this process, and accordingly colchicine was chosen as the first compound to be investigated. In 1949 Forman, Seifter and Ehrich[2] investigated the effect of colchicine and other drugs on the development of experimental serum disease in the rabbit, and reported that there was a small drop in the circulating antibody in the animals injected throughout the course of the experiment with full doses of colchicine. Fagræus and Gormsen[3] studied the effect of moderate to full doses of colchicine on antibody production in the rat, and demonstrated that when colchicine was administered on the third day following an injection of antigen, there was a statistically significant fall in the circulating antibody titer.

In our experiments, full doses of colchi-

cine were administered to rabbits at the same time as the second injection of antigen, and a pronounced increase in the amount of antibody present on the eighth day thereafter was demonstrated. The larger the dose of colchicine, the greater was the rise in antibody up to the point where the animals died from the drug. It has also been found that colchicine does not produce this effect when it is administered two days before or two days after the injection of antigen. In control experiments, it was found that colchicine alone does not produce a secondary response, in agreement with Fagræus and Gormsen. We did not investigate in a sufficient number of animals the effect of colchicine injected three days or longer after an injection of antigen, although what data we have are not inconsistent with the report of Fagræus and Gormsen.

No explanation of this effect of colchicine can be offered at the present time, although it is possible that colchicine stimulates the division of the primitive cells from which the antibody synthesizing cells spring. It was originally suggested by Dustin[4] and his collaborators that colchicine stimulated cell division. Later it was shown by Ludford[5] that the greatly increased number of mitotic figures visible during the first day after colchicine administration was due to the arrest of mitosis at the metaphase. More recently, however, Miszurski and Doljanski[6] have presented evidence that colchicine not only arrests mitosis, but also stimulates its occurrence. We have attempted to test this hypothesis by making cell counts of antibody containing cells in the regional lymph nodes of animals with and without colchicine treatment. Although so far our data show more antibody containing cells in colchicine-treated animals, the data are as yet too sparse to yield a statistically significant difference.

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